B2

11. (amended) The method of claim 1, wherein the LNP polynucleotide is operably linked to the promoter in sense orientation.

REMARKS

The Invention

The invention is the discovery that a lectin nucleotide phosphohydrolase (LNP) which binds nodulation factors of Rhizobium species, also promotes mycorrhizal associations between the plants and mycorrhizal fungi. The present application provides evidence that LNP functions at the initiation step of the plant-mycorrhizal interaction. Thus, LNP can be used to increase mycorrhizal infection in plants.

Status of the Claims

Claims 1-14 are pending in this application. Claims 2, 3, 6-8 and 10 are withdrawn from consideration and are cancelled without prejudice. Claims 1, 4, 5, 9, and 11-14 are rejected.

Claims 1, 4, 5, 8-9, and 11-14 are rejected under the judicially created doctrine of obviousness type double patenting.

Claims 1, 5, 9, and 11-14 are rejected under 35 U.S.C. §112 first Paragraph as containing subject matter not described in such a way as to convey that the inventors had possession of the invention at the time the application was filed.

Claims 1, 4-5, 8-9, and 11-14 are rejected under 35 U.S.C. §112, first Paragraph because they are allegedly not enabled for their full scope.

Claims 1, 4-5, 8-9, and 11-14 are further rejected under 35 U.S.C. §102(b) as being anticipated by Etzler et al. (1999, WO99/07223).

Claims 1, 11, and 12 are rejected under 35 U.S.C. §112, Second Paragraph for failing to point out and distinctly claim the subject matter the Applicants regard as their invention.

The Amendments

Support for the amendment to claim 1 is found in original claim 12 wherein it is stated that: "expression of the LNP polynucleotide is enhanced thereby increasing infection of the plant by a mycorrhizal fungus". The expression "is enhanced thereby increasing" is defined on page 14 of the specification, lines 11-13. Enhanced expression means to: "Increase expression of an endogenous gene or provide LNP expression in a plant that does not normally express LNP". Support for the additional step of selecting plants with a regulated mycorrhizal infection is found in the specification on page 26, lines 14-28 wherein the Applicants disclose methods for testing the ability of transgenic plants to be colonized by mycorrhizae. No new matter is added.

Support for the amendment to claim 11 is found in the original claim and on page 4, lines 12-14 wherein the term "operably linked" is defined. No new matter is added.

The Rejections

Double Patenting

The Examiner provisionally rejected claims 1, 4, 5, 8-9, and 11-14 under the judicially created doctrine of obviousness type double patenting as being unpatentable over claims 16-17, 20-22, 49-50, and 52 of copending application 09/129,112 (now U.S. Patent 6,465,716).

To address the Examiner's concerns claim 1 has been amended to recite an additional step of selecting plants with a increased mycorrhizal association. This limitation is not present in the cited reference and so the amendment should place claim 1 and its subsequent dependent claims 4, 5, 8-9, and 11-14, in condition for allowance.

Rejections under 37 U.S.C. §112 First Paragraph

Written description

The Examiner rejects claims 1, 5, 9, and 11-14 under 35 U.S.C. §112 for alleged inadequate written description. Applicants respectfully traverse the rejections. The Examiner asserts that the Applicants do not identify structural features unique to the LNP protein, the functional domains of the protein, nor the overall function of the protein and so alleges that the specification contains subject matter that was not described in such a way as to convey that the inventor had possession of the claimed invention at the time the application was filed.

To satisfy the requirement of written description, an application must describe the claimed invention in sufficient detail that one skilled in the art can reasonably conclude that the inventor had possession of the claimed invention. MPEP §2163 Sec. I. On the other hand, there is a strong presumption that an adequate written description of the claimed invention is present when the application is filed and the Examiner has the initial burden to present evidence or reasons why persons skilled in the art would not recognize in the disclosure a description of the invention defined by the claims. MPEP §2163 Sec. I. A. It is well established that "every species in a genus need not be described in order that a genus meet the written description requirement." *Utter v. Hiraga*, 6 USPQ 2d 1709, 1714 (Fed. Cir. 1989). As explained in the MPEP, the written description may be met through description of a representative number of species...or by disclosure of relevant identifying characteristics i.e., structure or other physical and/or chemical properties, by functional characteristics coupled with a known or disclosed correlation between function and structure, or by a combination of such identifying characteristics, sufficient to show the applicant was in possession of the claimed genus. MPEP §2163 Sec. II.A.3.(a)(ii).

As explained below, the rejection must fail because the genus of nucleic acid molecules recited in the claims is defined by a representative number of species and because common structural features correlated with function have been provided.

In the first paragraph of the present application, Applicants incorporate by reference USSN 09/129,112, filed August 4, 1998. The '112 application issued as U.S. Patent No. 6,465,716. U.S. Patent No. 6,465,716 discloses characteristic features and functions of the LNP isolated from the legume *Dolichos biflorus*. The patent also discloses the isolation of genes encoding the *Lotus* and *Medicago* LNP proteins using degenerate primers to conserved sequences of *Dochilos biflorus* LNP (U.S. Patent No. 6,465,716 column 22, lines 21-39).

The inventors have published a peer reviewed article discussing the shared structural features and functions of these proteins (Roberts *et al.* (1999) *Mol Gen Genet* 262:261-267, attached as Exhibit 1) There, the authors discuss the relationships of the *Dolichos*, *Medicago,* and *Lotus* genes. In particular, based on sequence analysis of these and other genes, the authors conclude that these three genes are orthologs (*i.e.* are genes that perform the same or similar function) and that other genes from non-leguminous plants (*e.g.*, *Arabidopsis*) are not related in that way (*see*, *e.g.*, Abstract, page 261, and Discussion, page 267). Thus, this publication shows that, based only on sequence analysis, those of skill reasonably conclude that all three disclosed members of the claimed genus function in the same way in plants.

All of the LNPs of the present invention comprise four conserved sequence motifs characteristic of apyrase enzymes (U.S. Patent No. 6,465,716 column 16, lines 57-59). These motifs are shown in the specification in the figure illustrating *Dolichos biflorus* LNP, SEQ ID NO:2. The conserved motifs are boxed in the figure and are easy to identify in SEQ ID NOs:4 and 10. Thus, the LNP proteins all share conserved sequence motifs and activity that identify them as an apyrase enzyme.

However, unlike other apyrases, the LNPs of the invention have and additional identifying characteristic: they also have carbohydrate binding activity. As disclosed in U.S. Patent No. 6,465,716 column 15, lines 43-47, LNP can be isolated from *Dolichos biflorus* root extracts using hog gastric mucin blood group A + H substance conjugated to sepharose. Furthermore, competition experiments with blood group substances and oligosaccharides (U.S. Patent No. 6,465,716, column 15, lines54-67 and

column 16, lines 1-14) indicate that LNP proteins are Nod factor binding proteins (U.S. Patent No. 6,465,716, column 16, lines 26-41). Indeed, the properties of *Dolichos biflorus* LNP suggest that LNP may play a role in the initiation of Rhizobium-legume symbiosis (Roberts *et al.* (1999) *Mol Gen Genet* 262:261-267, discussion section, page 266).

As noted above, adequate written description of the invention may be shown by description of a combination of sufficient, relevant, identifying characteristics. For some biomolecules examples of identifying characteristics include sequence, structure, binding affinity, binding specificity, molecular weight and length MPEP 2163 II. 3 (a). The Applicants have elucidated the features of LNPs that identify them as apyrase enzymes. They have further identified structures and functions such as carbohydrate binding, that distinguish LNP from other known apyrases, and finally, have identified a specific function for the protein in nod factor binding and the initiation of symbiosis. These same conclusions have been published in a peer reviewed journal. To maintain this rejection, the Examiner must explain how the application fails to meet the written description requirement of the patent laws in light of these facts. In the absence of evidence or reasoning to support the rejection, Applicants respectfully request that the rejections for lack of written description be withdrawn.

Enablement

The Examiner rejects claims 1, 4-5, 8-9, and 11-14 under 35 U.S.C. §112, first paragraph as allegedly not being enabled for the broad scope of modulating mycorrhizal infection in any plant. Applicant traverses the rejections.

To satisfy the enablement requirement, an application must contain sufficient information regarding the subject matter of the claims so as to enable one skilled in the art to make and use the claimed invention. MPEP §2164.01. The test for enablement is set forth in *In re Wands*, 858 F.2d 731, 8 USPQ 2d 1400 (Fed. Cir. 1988), and requires consideration of multiple factors including: the breadth of the claims; the nature of the invention; the state of the prior art; the level of predictability in the art; the

amount of direction provided by the inventor; the existence of working examples; and the quantity of experimentation needed to make or use the invention based on the content of the disclosure.

In the present case, the claims are directed to methods of using nucleic acids encoding proteins with a defined structure and readily testable activity. The specification also contains ample directions to practice the invention, such as methods of cloning nucleic acid sequences (see, *e.g.*, page 12, line 31 to page 14, line 9), plant transformation and selection of plants with a modified mycorrhizal infection (see, *e.g.*, page 25 line 14 to page 26 line 28), and assays for activity (see, *e.g.*, U.S. Patent No. 6,465,716 column 16, line 63 through column 17, line 28). The Examiner has provided no evidence to show that any of these methods are unpredictable or difficult to carry out for one of skill in the art.

The Examiner raised the concern that there was insufficient guidance for one skilled in the art to practice the invention, because the specification allegedly does not show which amino acids of SEQ ID NO:10 can be altered and how, and which amino acids must not be changed in order to maintain activity of the protein. The Examiner further alleges that the state of the art provides no guidance as to which domains are required to maintain proper enzymatic or receptor activity. Applicant disagrees with these assertions.

As noted above, the LNP proteins of the invention comprise four conserved structural motifs characteristic of apyrase enzymes. Since apyrase activity is associated with the function of the proteins in signal transduction, these regions of the proteins are relatively sensitive to modification. Furthermore, the carbohydrate binding domains of the protein are related to the recognition of the mycorrhizal fungus and initiation of symbiosis. The Applicants have provided ready assays to measure carbohydrate binding and apyrase activity (see e.g. U.S. Patent No. 6,465,716 column 15, line 40 through column 16 line 41 and column 16 line 63 through column 17, line 28). These assays can be used alone or in combination to distinguish LNPs from among those

proteins with 70% homology to SEQ ID NO:10. A skilled artisan who has mastered molecular biology and protein biochemistry will know how to identify proteins with relevant stretches of homology, and will know how to assay the selected proteins. The techniques required for the procedures, such as recombinant DNA technology, are well established and routinely used by those skilled in the art. Therefore, there would be no undue experimentation required to distinguish LNPs from all proteins with 70% homology to SEQ ID NO:10.

In the rejection, the Examiner also questions whether LNP proteins can be used to promote mycorrhizal infection in plants. The experiments reported in the present application demonstrate that antisense expression of the *Lotus japonicus* LNP eliminates mycorrhizal infection of *Lotus japonicus* by *Glomus intraradices* and prevents nodulation by *Mesorhizobium loti*. As explained below, this evidence in combination with knowledge in the art, shows that LNP is a shared component of the pathways required for the establishment of both mycorrhizal association and Rhizobial infection.

Attached as Exhibit 2 is a copy of a peer reviewed publication, Albrecht *et al.* (1999) *EMBO Journal* 18:281-288. The article is cited in the specification on page 2, lines 16-17, and page 21, lines 28-29 and 34. The article shows that:

(1) the pathways leading to mycorrhizal-plant symbiosis and Rhizobium-legume association use the same gene products, which suggests that the pathways have common steps (*see*, page 285 second column, paragraph 5), and

(2) the genetic pathways leading to the establishment of mycorrhizal or rhizobial infection are conserved, even in plants that do not normally manifest such symbiotic interactions (page 287, first column, second paragraph). Thus, the article makes clear that one of skill in the art could expect the *initiation* signal from one plant to be able to promote mycorrhizal or rhizobial infection in any plant into which such a signal is introduced.

In conclusion, as noted above, Roberts *et al.* establishes that those of skill in the art recognize LNP proteins are structurally related and function to promote nodulation in legumes. Roberts *et al.* also suggest that LNP may play a role in the

initiation of rhizobium-legume symbiosis. The Albrecht *et al.* article shows that those of skill further recognize that nodulation and mycorrhizal infection use the same gene products and that the genetic pathways leading to the establishment of mycorrhizal or rhizobial infection are conserved among plants.

The art, however, lacked evidence that, in fact, LNP acted as the recognition protein in mycorrhizal infection, as well as nodulation. This evidence is provided in the present application. As noted above, the present application shows that antisense expression of one member of that genus (*Lotus japonicus* LNP) eliminates mycorrhizal infection and prevents nodulation in transgenic plants. LNP thus functions at the initiation step of the plant-mycorrhizal and plant-Rhizobium interactions. Since pathways leading to plant-Rhizobium, plant-mycorrhizal symbiosis overlap, and since the signal cascade leading to symbiosis is conserved in the plant kingdom, the lectin nucleotide phosphohydrolase (LNP) gene can be transformed into any plant to promote mycorrhizal associations between the plant and mycorrhizal fungi.

In light of this evidence, the Examiner must provide reasoning or evidence that one of skill would not be able to practice the claimed invention without undue experimentation. In the absence of reasoning or evidence that refutes the above, the rejection is improper and should be withdrawn.

Rejections under 35 U.S.C. §102(b)

The Examiner rejects claims 1, 4-5, 8-9, 11-14 under 35 U.S.C. 102(b) as being anticipated by Etzler et al. WO99/07223. The Examiner states that because the method steps and the LNP nucleotide sequences are the same, the ability to modulate mycorrhizal infection is inherent in the reference.

The claims have now been amended to include the additional step of selecting plants with a increased mycorrhizal association. This limitation is not present in the cited reference and so the amendment should place the claims in condition for allowance.

Rejections under 35 U.S.C §112 second paragraph

Claims 1, 11, and 12 are rejected for failing to particularly point out and distinctly claim the subject matter the Applicant regards as the invention.

Claim 12 has been canceled Claims 1 and 11 have been amended to overcome the Examiner's rejections, and the limitations of claim 12 have been incorporated into claim 1.

Claim 1 has been amended to recite "increasing" in the place of "modulating" so that it should now be clear that introducing into a plant an LNP operably linked to an expression cassette into a plant will promote mycorrhizal association of a plant and a mycorrhizal fungus. Thus the limitations of claim 12 have been incorporated into claim 1. The Examiner's rejected of claim 12 for indefiniteness because the Examiner alleged the metes and bounds of the term "expression of the LNP polynucleotide is enhanced" were unclear. On page 14 of the specification, lines 11-13 the Applicants define specifically what is meant by the term enhanced expression. To enhance expression means to: "Increase expression of an endogenous gene or provide LNP expression in a plant that does not normally express LNP". Therefore the metes and bounds of the term "expression of the LNP polynucleotide is enhanced" are clear.

Claim 11 has been amended to recite "wherein the LNP polynucleotide is operably linked to the promoter in the sense orientation" instead of "wherein the promoter is linked to the LNP polynucleotide in a sense orientation". The Applicants thank the Examiner for his kind suggestion as to how the claim could be amended.

CONCLUSION

In view of the foregoing, Applicants believe all claims now pending in this Application are in condition for allowance. The issuance of a formal Notice of Allowance at an early date is respectfully requested. If a telephone conference would

expedite prosecution of this application, the Examiner is invited to telephone the undersigned at .

Respectfully submitted,

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VERSION WITH MARKINGS TO SHOW CHANGES MADE

1. A method of [modulating] increasing mycorrhizal infection in a plant, the method comprising introducing into the plant an expression cassette containing a plant promoter operably linked to a heterologus LNP polynucleotide or complement thereof, wherein the LNP polynucleotide encodes an LNP polypeptide at least about 70% identical to SEQ ID NO:2, SEQ ID NO: 4, or SEQ ID NO: 10, and selecting plants that have increased mycorrhizal infection.

11. (amended) The method of claim 1, wherein the LNP polynucleotide is operably linked to promoter [is linked to the LNP polynucleotide] in [a] sense orientation.